Characterization of Gene Alterations following Editing of the beta-Globin Gene Locus in Hematopoietic Stem/Progenitor Cells.

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Funding Grants: Beta-Globin Gene Correction of Sickle Cell Disease in Hematopoietic Stem Cells

Public Summary:
Gene editing of the beta-globin gene to treat sickle cell disease may be complicated by co-editing at the closely related delta globin gene. Cutting by nucleases (like CRISPR or zinc finger nucleases) at both beta- and delta-globin can lead to chromosomal rearrangements. These types of complications need to be assessed in pre-clinical studies to enure patient safety.

Scientific Abstract:
The use of engineered nucleases combined with a homologous DNA donor template can result in targeted gene correction of the sickle cell disease mutation in hematopoietic stem and progenitor cells. However, because of the high homology between the adjacent human beta- and delta-globin genes, off-target cleavage is observed at delta-globin when using some endonucleases targeted to the sickle mutation in beta-globin. Introduction of multiple double-stranded breaks by endonucleases has the potential to induce intergenic alterations. Using a novel droplet digital PCR assay and high-throughput sequencing, we characterized the frequency of rearrangements between the beta- and delta-globin paralogs when delivering these nucleases. Pooled CD34(+) cells and colony-forming units from sickle bone marrow were treated with nuclease only or including a donor template and then analyzed for potential gene rearrangements. It was observed that, in pooled CD34(+) cells and colony-forming units, the intergenic beta-delta-globin deletion was the most frequent rearrangement, followed by inversion of the intergenic fragment, with the inter-chromosomal translocation as the least frequent. No rearrangements were observed when endonuclease activity was restricted to on-target beta-globin cleavage. These findings demonstrate the need to develop site-specific endonucleases with high specificity to avoid unwanted gene alterations.